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BEFORE THE BOARD OF PATENT APPEALS

AND INTERFERENCES

Paper No. 15

Application Number: 08/468,610

Filing Date: 06/06/95

Appellants: Burton et al.

Gerald Swiss

For Appellant

EXAMINER'S ANSWER

This is in response to appellant's brief on appeal filed 25 August 1997.

(1) Real Party in Interest

A statement identifying the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

A statement identifying the related appeals and interferences which will directly affect or be directly affected by or have a bearing on the decision in the pending appeal is contained in the brief.

(3) Status of Claims

The statement of the status of the claims contained in the brief is correct.

(4) Status of Amendments After Final

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

(5) Summary of Invention

The summary of invention contained in the brief is correct.

(6) Issues

The appellant's statement of the issues in the brief is correct.

(7) Grouping of Claims

Appellant's brief includes a statement that claims 1-5 and 7-23 do not stand or fall together and provides reasons as set forth in 37 CFR 1.192(c)(7) and (c)(8).

(8) Claims Appealed

The copy of the appealed claims contained in the Appendix to the brief is correct.

(9) Prior Art of Record

The following is a listing of the prior art of record relied upon in the rejection of claims under appeal.

Sasaki et al. (1979) "Hydrophobic-Ionic Chromatography, Its Application to Purification of Porcine Pancreas Enzymes", **J. Biochem.**, vol. 86, pages 1537-1548.

Sasaki et al. (1982) "Hydrophobic-Ionic Chromatography, Its Application to Microbial Glucose Oxidase, Hyaluronidase, Cholesterol Oxidase, and Cholesterol Esterase", **J. Biochem.**, vol. 91, pages 1555-1561.

Kasche et al. (1990) "Rapid Protein Purification Using Phenylbutylamine-Eupergit: A Novel Method for Large-Scale Procedures", **J. Chromatog.**, vol. 510, pages 149-154.

Jost et al. (1974) "The Mode of Adsorption of Proteins to Aliphatic and Aromatic Amines Coupled to Cyanogen Bromide-Activated Agarose", **Biochim. Biophys. Acta**, vol 362, pages 75-82.

Teichberg, V.I. (1990) "Affinity-Repulsion Chromatography", **J. Chromatog.**, vol. 510, pages 49-57.

(10) New Prior Art

No new prior art has been applied in this examiner's answer.

(11) Grounds of Rejection

The following grounds of rejection are applicable to the appealed claims:

Claims 1-5 and 7-23 stand rejected under 35 U.S.C. § 103 as being unpatentable over Sasaki et al. (1979) or Sasaki et al. (1982) in view of Kasche et al. (1990), Teichberg (1990) and Jost et al. (1974).

A complex between an ion exchange resin and a target protein is claimed wherein the complex is formed at a pH value of between 5-9, where the resin is uncharged, and the target protein is bound to the resin by hydrophobic interactions. The ion exchange resin consists of a solid support matrix and a covalently attached ionizable ligand.

Sasaki et al. (1979) disclose binding several enzymes onto Amberlite CG-50 at a pH value of 4.0 where the carboxyl groups are not dissociated and, consequently, the Amberlite is uncharged. The resin can be eluted by increasing the pH so that the carboxyl groups dissociate with a concomitant loss of hydrophobicity and acquisition of a repulsive charge which in combination decreases the binding affinity of the bound enzymes. This process of using the Amberlite ion-exchange medium is termed hydrophobic-ionic chromatography. At page 1548, the hydrophobic-ionic type of chromatography is defined as when the order of elution of proteins from the resin "is controlled by the remaining hydrophobic affinity *plus* the increased electrostatic affinity *minus* the increased electrostatic repulsion produced as the carboxyl groups are dissociated." Contrary to conventional ion exchange chromatography, enzymes bind to the uncharged functional groups and dissociate when the functional groups become charged.

The acid base titration curve of Amberlite CG-50 shown in Fig. 1 demonstrates that below a pH value of about 4.5, the resin is fully protonated and therefore should exhibit no

charge. Additionally, this figure demonstrates to a person of skill in the art how to determine the effective pH range of a given resin in the hydrophobic-ionic chromatography method. That is, the titration curve shows conditions when the protein will be bound by hydrophobic effects, and conditions when ionic effects will dissociate the protein.

The lack of ionic strength dependence on the binding of proteins at pH 4 to Amberlite CG-50 (page 1540, column 2) and elution of proteins with organic solvents (page 1546, column 2) is further evidence that the enzymes are bound to the ion exchange matrix by hydrophobic effects. Sasaki et al. (1979) lack forming the complex with a resin that is uncharged between pH values of 5-9.

Sasaki et al. (1982) disclose binding several microbial enzymes onto Amberlite CG-50 at a pH value of 4.0 where the carboxyl groups are not dissociated and, consequently, the Amberlite is uncharged. Subsequently elution is effected by increasing pH to ionize the resin. This overall process is termed hydrophobic-ionic chromatography (abstract). In Figure 5 a cartoon is provided to explain the proposed mechanism of hydrophobic-ionic chromatography. The cartoon clearly indicates, **in general terms**, that with an acidic group, binding occurs below a certain pH and desorption occurs above the critical pH. Different proteins having different interacting groups are released at different critical pH values (X or Y).This cartoon does not require any particular resin. It is a generalization of the concept of hydrophobic-ionic chromatography. The figure legend to Figure 5 clearly states, "**in the case of** Amberlite CG-50, X is 4.5" (emphasis added by examiner). The figure legend continues to describe general mechanism, "with the use of **appropriate adsorbent** carrying alkaline

groups, ... the relationship to pH would be opposite". While the cartoon illustrates the general principle with an ion-exchange resin that is acidic in nature, if the general mechanism is applied to an ion-exchange resin which is basic in nature, the effect of pH would be the opposite, i.e, lowering the pH would effect elution as the basic resin became charged. To understand this better, consider a resin with an amine functional group attached thereto. At sufficiently alkaline pH values, the resin is in the form of free amine and is uncharged. At lower pH values the basic amine becomes protonated to its conjugate acid and assumes a positive charge. Sasaki et al. (1982) lack forming the complex with a resin that is uncharged between pH values of 5-9.

Kasche et al. (1990) disclose binding proteins to phenylbutylamine-Eupergit and subsequently decreasing the pH to elute and purify proteins. At page 150, the hydrophobic-ionic chromatography method is described; proteins are bound primarily by hydrophobic interactions at one pH, and eluted by electrostatic repulsion at a different pH. It is remarked that what is needed is a pH value where the electrostatic repulsions are stronger than the hydrophobic adsorption. In Kasche et al., the resin ligand is a basic amine group so that at lower pH, the group becomes protonated and charged (see Sasaki et al., 1982). In Figure 2, the titration of the ion exchange resin is shown. At pH values of about 9 and above, the resin is said to bind protein by hydrophobic interactions. At lower pH values the protein is desorbed by electrostatic repulsion.

Teichberg (1990) disclose affinity-repulsion chromatography to elute proteins bound to an affinity chromatography matrix. The method is based upon the principle that both the protein and the affinity matrix possess ionizable charges which can attract or repel each other. The repulsion

can be effected and the protein eluted from the affinity matrix under conditions when the strength of electrostatic repulsion is greater than the attraction between the protein and the immobilized ligand (page 50). This is further illustrated in the cartoon of Figure 1.

Jost et al. (1974) discloses that even negatively charged proteins can be bound to agaroses having aliphatic and aromatic amines coupled thereto by cyanogen bromide activation. A mixture of ionic and hydrophobic effects were seen with these ligands. The hydrophobic effects were said to result primarily from the spacer arms, whereas the ligands are charged. It was thought that the spacer/ligand acted like a detergent. A single pH value of 8.0 was used to form the complex between the matrix and protein.

A person of ordinary skill in the art at the time the invention was made would have been motivated to form a complex between proteins and an uncharged ion exchange matrix at a pH value of between 5-9 from which the protein can be eluted by changing the pH value because Sasaki et al. (1979) and Sasaki et al. (1982) teach the general principle of binding proteins to uncharged ion exchange resins by hydrophobic effects and the subsequent elution of the bound proteins by a change in pH, and because of the generally recognized stability of proteins near physiological pH values of 5-9. The selection of pH values where the ion-echange resin is uncharged is an arbitrary matter of experimental design choice.

Sasaki et al. (1982) admit that a limitation of the exemplified Amberlite CG-50 is that it can only be used at acidic pH values below 4.5, pH values which may be problematic with proteins that are not stable at such acidic pH values. While Sasaki et al. (1982) realize the limits of the specific resin that they used, they suggest the general mechanism of the

hydrophobic ionic chromatography method and even suggest the use of alternative "appropriate" functional groups on the resin to obtain other pH regimes.

The disclosure in Sasaki et al. (1982) provides ample motivation for a person of ordinary skill in the art to select other "appropriate groups" on the resin when the protein to be purified by this method is not stable at acidic pH values. A person of ordinary skill in the art would either know of the pH stability of the protein that they want to purify, or they could readily determine the pH stability with nothing more than routine experimenal methods known to those of ordinary skill in the art.

Kasche et al. (1990) extends the method of Sasaki et al. to basic resins (as suggested in the Figure 5 legend of Sasaki et al., 1982). Kasche et al. also confirms the binding and subsequent elution of bound proteins in pH regimes where the resin already has a partial charge. The titration curve shown in Kasche et al. demonstrates a recognition of hydrophobic binding at high pH values, and electrostatic repulsion at low pH values. The fact that Kasche et al. use a pH value of 8.0 for their purification illustrates their contention that it is only at pH values where electrostatic repulsion exceeds the strength of hydrophobic binding that the proteins will be eluted. The interpretation of this observation by a person of ordinary skill in the art is that different proteins have differential affinity for the ion exchange matrix in the pH regime where the ionizable groups of the matrix are being titrated. That is, the teachings of Kasche et al. confirm that the pH regime where hydrophobic ionic chromatography may be performed is not limited to pH values where the resin is totally uncharged. Both Sasaki et al. and Kasche et al. recognize that it is the sum of attractive and repulsive forces that determine

protein binding to the matrix. These forces also result from hydrophobic and ionic groups on the protein which are titrating as the pH values are changed.

Teichberg (1990) and Jost et al. (1974) provide further evidence that the method can be expanded to include other ligand/matrix combinations where repulsive ionic interactions can be used to elute proteins from an attached ligand.

The selection of a resin from among well-known chromatography resins for use in hydrophobic ionic chromatography where the resin is uncharged in the range of pH of 5-9 involves nothing more than routine experimentation to determine the titration curve of the resin. The selection of any given pH dependency for the resin will depend on the stability of the protein to be purified and the isoelectric point of the protein, parameters which are readily determined by the person of ordinary skill in the art or are already known to them. Sasaki et al. and Kasche et al. provide specific resins which are uncharged just outside the pH range claimed. Resins are known in the art which would be uncharged within the pH range of the scope of the claims.

Hence, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to substitute other ion-exchange resins with appropriate functional groups such that the resins were uncharged in the pH range of 5-9 for the resins of Sasaki et al. and to form a complex between the ion exchange resin and a desired protein as at least a step in the purification of the protein. Other uses of bound proteins are known in the art.

(12) New Ground of Rejection

This examiner's answer does not contain any new ground of rejection.

(13) Response to argument

It is argued that the claimed invention is directed to a composition. It is urged that *in re Vaeck* and *ex parte Stauber and Eberle* require that 1) the prior art suggest the claimed composition, 2) the prior art provides a reasonable likelihood of success, 3) there is some logical basis for combining the prior art references - motivation - so as to obtain the claimed invention. It is urged that *in re Deuell* requires consideration of similarity to prior art compositions. Accordingly it is urged that a *prima facie* case has not been established over the instantly amended claimed compositions. Specifically, it is argued that Sasaki et al. do not provide motivation to extend their method to pH values between 5-9 and that opposite the charges discussed in Figure 5 would provide for pH values greater than 9.5. Kasche et al. is said to fail because the resin retains substantial electrostatic charge at pH of 8.0 where the protein purification was actually performed. It is urged that Teichberg and Jost et al. are irrelevant to the claims because these references fail to address the problem of the claimed invention. It is argued that the allegation in the Advisory action that proteins can still bind to the resin when there is partial charge is not germane to the claimed invention where the resin is uncharged.

The cartoon in Sasaki et al. (1982) clearly conceptualizes the general principle of hydrophobic ionic chromatography. The cartoon itself makes no reference to any particular resin. It is explained that the first pH value X is the highest pH where the proteins are bound by strictly hydrophobic effects. The pH value of Y is the lowest pH where hydrophobic effects have been completely overwhelmed by ionic repulsions. At pH values between X and Y, the proteins are seen to differentially dissociate from the matrix. This is entirely consistent with the mechanism of hydrophobic ionic chromatography, wherein the order of elution of proteins from the resin "is

controlled by the remaining hydrophobic affinity *plus* the increased electrostatic affinity *minus* the increased electrostatic repulsion produced as the carboxyl groups are dissociated." This concept is repeated by Kasche et al. who state that the proteins will elute from the matrix when the electrostatic repulsions are stronger than the hydrophobic repulsions.

The interactions between the protein and the matrix are not simple. The protein itself has ionizable functional groups and hydrophobic groups on its surface. Changes in pH will change the net electrostatic charge on the matrix and the protein. Hence, it is more correct to speak of the sum of the hydrophobic and electrostatic interactions as do Sasaki et al. and Kasche et al. The elution of protein from the matrix as the pH is changed will be intermediate in pH regions where a partial charge is available. This will be in a pH region near the dissociation constant for the functional group on the matrix and can roughly be approximated by the Henderson-Hasselbach equation for acid dissociation. At the same time that the matrix acquires a charge with a change in pH, it is expected that the charge of the protein will also change. Thus charge-charge attractions as well as repulsions will occur. Any person of ordinary skill in the art is aware of these ramifications and will be able to select a resin which maximizes repulsion over attraction.

The direction of pH change and the selection of resin depends on the isoelectric point of the protein to be purified. At the isoelectric point the protein is uncharged. At pH values below the isoelectric point, the protein is positively charged and experiences primarily repulsion if the matrix acquired a positive charge with a decrease in pH. Similarly at pH values above the isoelectric point, the protein will be negatively charged and would experience primarily repulsion if the matrix acquired a negative charge with an increase in pH. The selection of an appropriate

ion-exchange resin is essentially the opposite reasoning that would be applied to selecting a resin where it was desired to bind the protein by ionic attractions as in conventional ion-exchange chromatography.

It is not irrelevant that Sasaki et al. and Kasche et al. point out regions of pH where there is a partial charge on the matrix. The resin is expected to form a complex even when there is a partial charge. Kasche et al., Teichberg, and Jost et al. were cited as evidence of this awareness and the ease with which the useable pH range of a given resin can be determined. The mere fact that protein binds at a pH where some charge is on the resin does not discredit their disclosure of the pH range where the resin ionizes. Kasche et al. recognize that the matrix can be used to bind proteins even when the resin is partially charged. Rather than teaching away from the claimed invention, this disclosure extends it. As discussed above, this is an extension of the cartoon described by Sasaki et al. (1982). In the pH regime between X and Y, the protein represented by the triangle is still bound to the resin. Kasche et al. confirms the prediction of Sasaki et al. that a protein can still bind to the resin which is partially charged. The differential affinity of proteins for the resin at different extents of resin/protein charge resulting from changes in pH is the basis for further purification as is well known in the art of protein purification and which is amply discussed in the technical literature provided by the manufacturers of ion exchange resins.

Since it is a composition that has been claimed, the question is whether it would have been *prima facie* obvious to use a different matrix with a different range of pH where the matrix is uncharged. Given the general mechanism conceptualized by Sasaki et al. it is urged that a person of ordinary skill in the art is aware of and would be able to select an appropriate ion-exchange resin having functional groups which ionize such that the ion-exchange matrix would remain

uncharged in the pH range claimed. There is a more than a reasonable likelihood of success in using resins which ionize at other pH values compared to Sasaki et al. or even to use pH values where the resin is partially charged as predicted by Sasaki et al. and demonstrated by Kasche et al. The claimed complex involves only recognition of the pH stability range of the protein and the protein's isoelectric point, parameters which should be known or readily measured by the person of ordinary skill in the art. These requirements are suggested by the prior art and nothing more than routine application of the teachings of Sasaki et al. is required to provide a protein/ion exchange complex which is uncharged in the pH range of 5-9.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

J.P.W. 24 November 1997

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